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428702
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THE VITALITY OF CELL FRAGMENTS OF YEAST

IV. The Relation of the Nucleus to Growth Ability

TRANSLATION NO.
808

JUNE 1963

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

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Folia Biologica, Vol. 2, 1956, pp. 29-35 and plates IX and X.

Translated by Sp/6 Charles T. Ostertag Jr.

The Vitality of Cell Fragments of Yeast.

IV. The Relation of the Nucleus to Growth Ability.

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Submitted to the Editor 19/IX 1955

In preceeding works we described (Necas, 1955 a, b, c) the evolutionary changes of plasmatic droplets of yeasts and the regeneration of cells. The present work is devoted to a detailed analysis of the relationship of the nucleus to growth processes and the regeneration of cell fragments.

To us, one of the most important problems is, in reality do only those droplets which contain a nucleus develop, or do droplets without a morphological nucleus have a growth capability. In the majority, facts from literature agree that only nuclear fragments of cells are capable of regeneration (for example, Klebs, 1887 a, b; Provazed, 1907; Lyncy, 1919; Haberlandt, 1919, 1929, and others). However there exists a whole series of works which very convincingly show that non nucleated fragments are capable not only of survival over a protracted period of time but of several regenerating processes and even growth. Thus Acqua (1891) and Palla (1889, 1890, 1906) observed the formation of a morphological cellular membrane in non nucleated cell fragments from higher and lower plants. Most important are Hammerling's precise experiments (1934 a, b, 1946) with Acetabularia. Their non nucleated parts not only grow but are also distinguished into almost normal plant parts. Brachet and Chantrenne (1951), with the help of labeled carbon, demonstrated that growth, stimulated by protein synthesis, was actually taking place here. The experiments of O. B. Lepishinskaya (1952), in which she attempted to demonstrate the possibility of nucleus origination in non nucleated plasmatic droplets from hydra cells, have not received confirmation up until now.

Methods

For the exposure of the yeast nuclei we used Feulgen's reaction with the following modifications: Fixation according to Carnoy, hydrolysis for 5-7 min. in 1/n HCl at 60 degrees, Schiff reagent for 2-3 hours. Staining with iron hematoxylin was conducted by Heidenhain's classical method. In phase contrast the nuclei of plasmatic formations are noticeable only infrequently.

Results

Dependence of the Growth of Plasmatic Droplets on the Presence of a Nucleus. Plasmatic droplets, originating as the result of the mechanical pulverization of cells, contain nuclei in a negligible number of cases. The reason for this is that the nuclei are destroyed by pressure earlier than the plasma is (as we demonstrated on the basis of the relationship between the overall volume of the plasmatic droplets and the overall number of nuclei - Necas, 1955), and also that the protoplast of a single cell (in our case - mononuclear) is crushed into a large number of fragments. In an autolysate, the plasmatic droplets contain nuclei far oftener, in approximately 60-70% of the cases. This is explained by the fact that all the cell nuclei survive but the protoplast breaks down into a small number of pieces, - and that in a culture subjected to autolysis, which is always no younger than several days, the non nucleate droplets perish thanks to which a relative amount of plasmatic droplets with a nucleus grow.

If a comparison is made of the percentage of plasmatic droplets with a growth capability from a mechanically homogenized mass and from a autolysate, than it turns out that in the first case only 0.5% of the plasmatic droplets develop while in the 2nd case it is 50-60% (based on a computation of approximately 2000 plasmatic droplets).

	% of plasm. droplets with a nucleus	% of plasm. droplets capable of devpt
From the homogenate	7	0.5
From the autolysate	60 - 70	50 - 60

In as much as it wasn't possible to simultaneously follow the development of the plasmatic droplets and to observe the nuclei, we had to be satisfied with the investigation of preparations fixed in the various periods of development of the plasmatic droplets. After inoculating the plasmatic droplets in a nutritive medium, we took samples every half hour and hour. In preparations stained according to Feulgen, there could be seen, first, non developing plasmatic droplets with a nucleus, secondly, non developing non nucleate formations, and finally, and this was most frequently, developing formations with a nucleus. In the course of the first 12 hours not one developing plasmatic formation without a nucleus was found.

From the facts presented a circumstantial deduction can be made that in the majority of cases only plasmatic droplets containing a nucleus develop.

Division of Nuclei in Plasmatic Formations. The nucleus in plasmatic droplets and in plasmatic formations during the beginning of their development, has the form of a completely normal nucleus of a yeast cell (fig. 1, 2, 3). The first division of the nucleus is observed 6-12 hours after reinoculation of the plasmatic droplet on a nutritive medium. In a 16 hour culture there are already approximately half of the plasmatic formations with 2 nuclei (graph 1, fig. 2). Formations with 3 nuclei begin to appear more ofter after

24 hours (fig. 3). However at this time there is already taking place a division of the formations themselves, which to a considerable degree distorts the picture of nuclei division. Very often a piece of plasma is cut off from the plasmatic formations which doesn't contain any nuclei at all. Often a small isolated section of plasma contains several nuclei while another large section has only one nucleus. In non dividing plasmatic formations, especially spherical ones, the number of nuclei increases constantly and after 48 hours may reach 8 - 10 (figs. 4, 5, 6, 7). The number of nuclei doesn't depend on the size of the plasmatic formations: Several comparatively small formations can be immediately packed with nuclei while other very large ones contain only several nuclei all together. As is apparent the nucleoplasma ratio is not maintained.

In the first phases of development of plasmatic formations, the nuclei, in preparations stained according to Feulgen, have a normal appearance. Later the stainability of nuclei by Feulgen's method is sharply lowered, the nuclei acquire considerable dimensions, indistinct contours and a pale-rose color. We think that this phenomenum is caused by the strong swelling of the nucleus. As a response to this there is a very low level of viscosity of the plasma in this stage of development of the plasmatic formations. In dense plasmatic formations the nuclei again take on the external appearance and stainability of normal cell nuclei. The nuclei of plasmatic formations stain very well with hematoxylin - much better than in normal yeasts. In several cases, with the help of a phase microscope, we succeeded in observing the nuclei in vivo. Their size, number, and disposition meet the essential qualities of fixed preparations stained according to Feulgen or with hematoxylin. (fig. 8).

On the basis of material available to us, we do not have the right to make conclusions relative to the mechanism of nuclear division in plasmatic formations. In scientific literature concerning the problem of the division of yeast nuclei there is a considerable non-uniformity of opinions (Schussnig, 1953). While observing plasmatic formations, we found those phenomena which defenders of the direct division of yeast nuclei consider as amitosis (fig. 10), and also phenomena which correspond to mitotic figures laid down by other authors. Very often the nuclear chromatin is concentrated in two elongated bodies, which Badian (1937), Subramaniam (1953) and Duraiswami (1953a, b) consider as chromosomes, while Levan (1946), Lindegren and Rafallko (1950), and Lietz (1957) consider them as clusters of chromosomes. In some plasmatic formations, especially with very long appendages, it is possible to clearly see, in addition to the nucleus, several Feulgen-positive granules lying freely in the plasma. They can be considered as stray chromosomes which Levan (1946) observed during atypical mitosis of yeasts under the influence of camphor and other factors. It is apparent from the chart that the frequency of nuclear divisions in plasmatic formations is much less than in yeast cells, in which there is an approximate 3-hour interval between divisions (Henneberg, 1926). The division of nuclei in plasmatic formations proceeds with time intervals of 6-12 hours, that is, 2-4 times slower. In product yeast cells, regenerated from plasmatic formations, the generation periods either remain the same length or are gradually drawn near to normal.

The nuclei in regeneration of cells from plasmatic formations. Dense plasmatic formations, out of which yeast cells form by means of budding, usually contain a considerable number of nuclei, which again appear like nuclei from normal yeasts. With the help of the Feulgen reaction, we succeeded several times in fixing directly regenerated formations. The first generations of cells are usually multinuclear (fig. 11A). Sometimes in each of these there are even more nuclei than in the original plasmatic formation (fig. 11B). In future generations of cells, the number of nuclei gradually decreases and finally single nucleated cells emerge. However, we isolated several strains of regenerated yeast cells in which multinuclear cells remained a permanent feature.

Discussion

Developing plasmatic formations contain a regular nucleus from the very beginning. We didn't find even one case of the development of a plasmatic droplet without a nucleus in spite of the fact that dozens of preparations with several thousand substances were studied. The greater the percentage of plasmatic droplets containing nuclei then the greater the number of them which are capable of growth. On the basis of these two facts the conclusion can be drawn that probably only those plasmatic droplets develop which contain nuclei. In one of our previous works (Necas, 1955c) we described the breaking up of individual pieces of plasmatic formations and the destruction of plasmatic droplets which became separated from them. It is very probable that these - namely such non nucleate pieces of plasmatic formations, are not capable of holding on long and are rudimentary. The results obtained by us up to now testify rather in favor of the view that the presence of a nucleus is necessary for protein synthesis and the processes of differentiation, and not in favor of the opinions of Hammerling (1946), Brachet (1951) and Lepeshinskaya. But it must be remembered that all these authors worked with completely different substances and that the results obtained for one substance can be transfered to other substances only with extreme caution.

In our last work we expressed the assumption that the restoration of metabolic processes in plasma, preceeding the morphological regeneration of cells, is already proceeding during the formation of dense plasmatic formations. Our new observations in reference to the fact that the nuclei in dense plasmatic formations, - in contrast to the nuclei of vacuolised plasmatic formations - appear as normal yeast nuclei, speaks in favor of this assumption.

In the majority of their morphological peculiarities, cells originating from plasmatic formations, correspond to normal cells. Therefore, it is fully understandable that there is ultimately set up in them a normal nucleoplasma relationship, that is one nucleus in one cell. It is extremely interesting however that multinuclearism, which develops during the growth of plasmatic formations, becomes a permanent peculiarity of several regenerated cells. The normal relationship between the nucleus and the plasma is not maintained here: The cells which are of normal overall dimensions contain several nuclei each. A similar heredity of artificially induced multinuclearism was already noted by Gerasimov (1902) and Van Wisselingh in Spirogyra.

In conclusion it must be emphasized that all of our observations relative to nuclei were made on fixed preparations, and we consider it a serious shortcoming. We didn't succeed in mastering the method of observing the development of nuclei in vivo, and in such a substance as yeast this is without a doubt very

difficult. Therefore, all of our arguments of the non capability of non nucleate cell fragments for development, we consider only as circumstantial arguments.

Summary

1. On the basis of inconclusive evidence the author comes to the conclusion that only those plasmatic droplets of yeast which contain nuclei are capable of development.

2. Plasmatic formations, originating as a result of the expansion of plasmatic droplets, contain 1 - 10 nuclei. The frequency of nuclear division is 2-4 times less than in normal cells. The form of division of the nucleus in some cases conforms to mitosis and in others - to amitosis. The nuclei are arranged in the plasma irregularly. The ratio between the nucleus and the plasma is not maintained. The division of plasmatic formations is apparently caused by physical reasons.

3. The first generation of cells, formed during the regeneration from dense plasmatic formations, are also multicellular. In future generations, the number of nuclei gradually decreases to one, however several strains of regenerated yeast cells firmly maintain the characteristic of multinuclearism. (Tables IX, X)

Chart 1. (page 30)

Axis x - growth of plasmatic formations in hours, Axis y - percentage formed with a specific number of nuclei. Line 1: 1 nucleus formation, 2: 2 nuclei, 3: 3 nuclei, 4: 4 nuclei, 5: 5 nuclei and multinuclear.

Plate IX

1. Plasmatic sphere approximately 6 hours after inoculation in nutritive medium. Beginning of growth. One nucleus in the center. Feulgen. Magnification 1000 X.

2. 12 hour plasmatic formations with long appendages. In the one on the right there is still one nucleus, forming two distinctly noticeable elongated bodies. In the left one, 2 nuclei are forming, one on each end. Feulgen. Magnification 1000 X.

3. Plasmatic formation with 3 nuclei. In several places there are vague contours of formation. Feulgen. Magnification 800 X.

4. 48 hour plasmatic formation with 5 nuclei. Feulgen. Magnification 1000 X.

5. Plasmatic formation with 4 nuclei. Hematoxylin. Magnification 1000 X.

Plate X

6. Another 48 hour plasmatic formation with 5 nuclei. Hematoxylin. Magnification 1000 X.

7. 60 hour plasmatic formation with lengthy appendages and with 7 nuclei. Hematoxylin. Magnification 1000 X.

8. Photograph of a plasmatic formation in a phase microscope. Several nuclei are noticeable. Magnification 1000 X.

9. Plasmatic formation with 3 nuclei concentrated in one place. Feulgen. Magnification 1000 X.

10. Elongated plasmatic formation. The division of the nucleus indicated by the arrow can be considered as amitosis. Hematoxylin. Magnification 1000 X.

Figure 11 (page 32)

A. Scheme of the number and arrangement of nuclei during regeneration of a plasmatic formation. Plasmatic formation with 4 nuclei in the center. The first generation is multinuclear, subsequently the number of nuclei decreases.

B. Regeneration of cells from a plasmatic formation. The first generation of cells contains more nuclei than the plasmatic formation.

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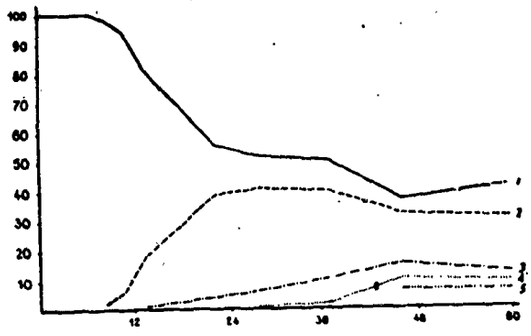


Chart 1. Explanation on page 5

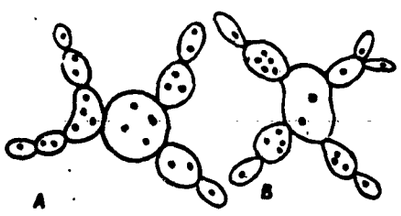
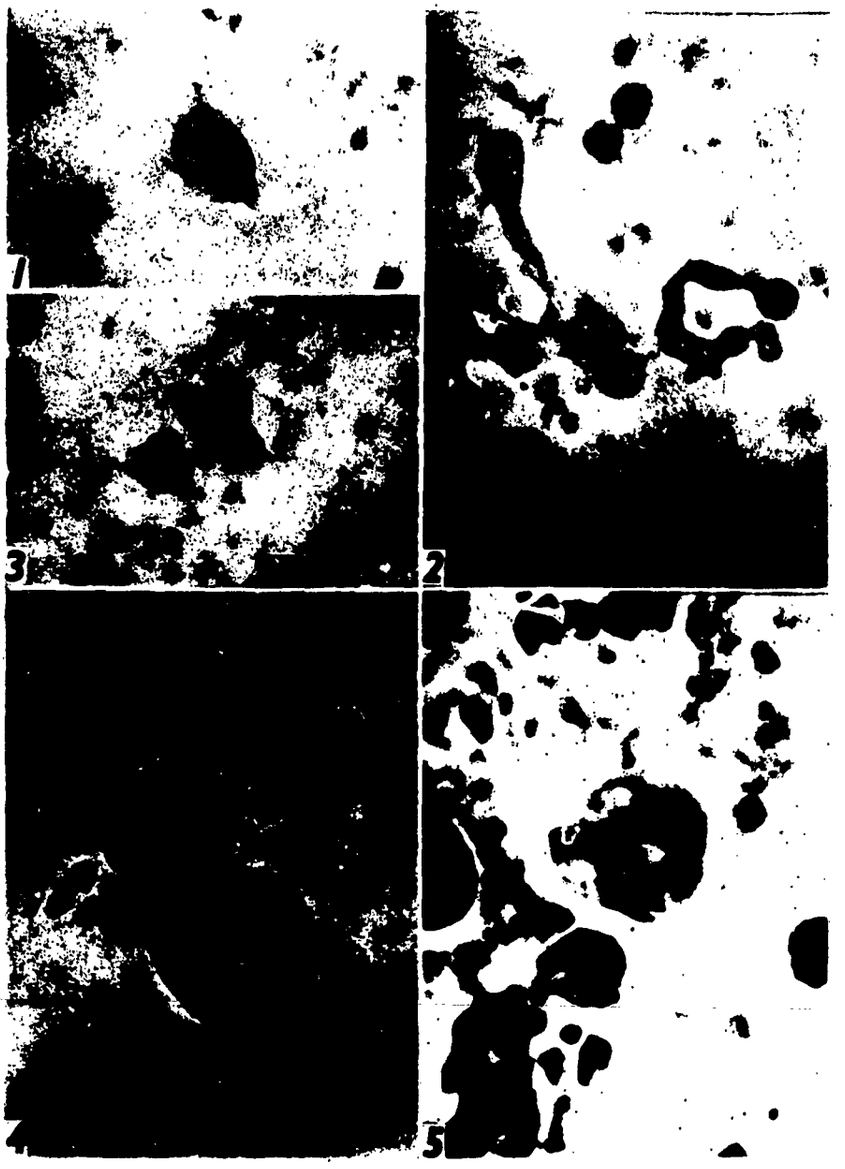


Figure 11. Explanation on page 6



Plates 1 thru 5 - Explanation on page 5

Plates 6 thru 10. Explanation on pages 5 and 6.